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Preliminary Studies on The Isolation of Pterins from the Wings of Heliconiid Butterflies[1]

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(Text-figures I-IV)

[This paper is presented as a portion of a series of studies on the Heliconiid butterflies which have been supported by the National Science Foundation and organized by Jocelyn Crane. The focal point of these studies has been the William Beebe Tropical Research Station of the New York Zoological Society at Simla, Arima Valley, Trinidad, W.I. The station was founded in 1950 by the Zoological Society's Department of Tropical Research under the late Dr. Beebe's direction.

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Introduction

PTERINS HAVE BEEN isolated from a variety of organisms, e.g., Tschesche & Vester (1955) isolated erythropterin from *Mycobacterium lacitcola,* Lecercq (1950) and Schöpf & Becker (1933) from Hymenoptera, Goto (1963) from Amphibia, and Forest & Mitchell (1954) from *Drosophilia,* to mention but a few. Pterins have also been isolated from the wings and eyes of various Lepidoptera (Pfleiderer, 1962; Schöpf & Becker, 1933). However, these studies have been limited to a few moths and butterflies of the family Pieridae. Essentially, this was due to the fact that it has been generally believed that pterins existed only within these groups of Lepidopterans (Ford, 1947; Ziegler-Günder, 1955). As a result of this study, however, it has been demonstrated that at least two Heliconiid butterflies contain pterins as their principle wing pigment.

Analysis of the red wing patches of *Heliconius erato adanis,* a black, neo-tropical butterfly with two distinct red spots, has led to the identification of erythropterin (Text-fig. I). Its chemical structure has been described by Purrman & Eulitz (1948), Fieser & Fieser (1963), and Tschesche & Korte (1951), and its properties by Fox (1953) and Albert (1954). A second pterin has been detected in the wings of the orange Heliconiid, *Colaenis julia,* but has not yet been identified.

Methods and Materials

The red pigmented regions of the wild caught *Heliconius erato* were removed. They were then defatted with ethyl ether in a Soxhlet apparatus, and the pigment extracted in a crude form with methanolic HCl, evaporated and redissolved in

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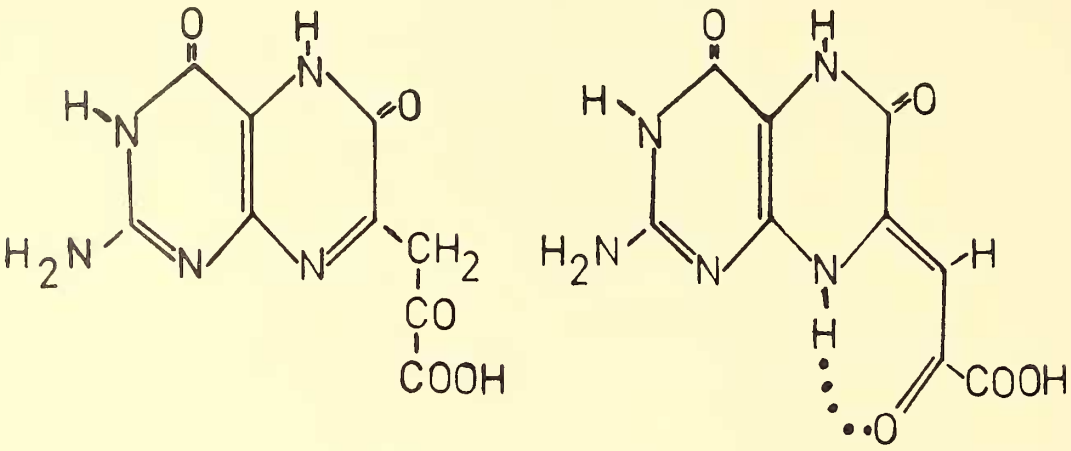


TEXT-FIG. I. The proposed bicyclic and tricyclic structures of erythropterin.

methanol. Since only minute quantities of the pigment were contained within the wing portions used, identification was initially limited to paper chromatographic techniques. Whatman filter paper #1 was used, and chromatograms were run in a butanol: acetic acid: water (4:1:5) solvent system.

Ultra violet and visible spectra of the above samples were recorded on a Beckman DK-2 Spectrophotometer.

RESULTS

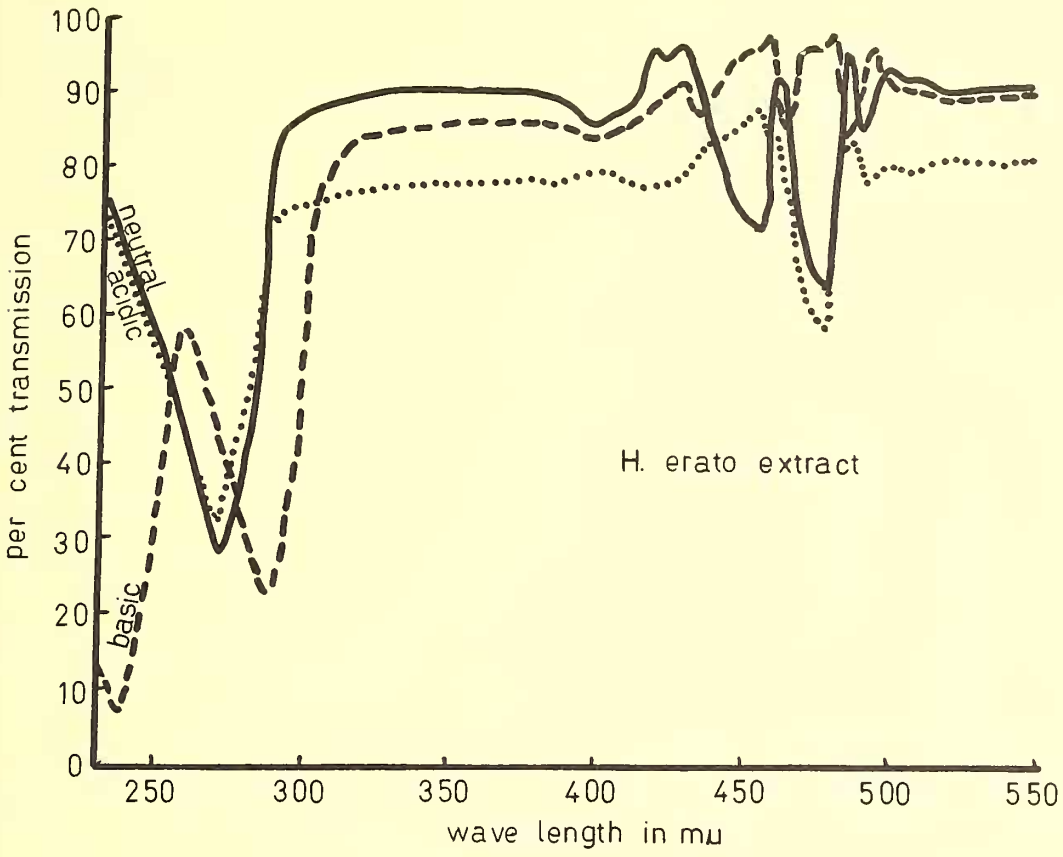
Initial experiments demonstrated that the physical and chemical properties of the pigment extract were consistent with those commonly attributed to pterins (Cromartie, 1958). The pigment was found to be insoluble in cold water and most organic solvents, was degraded by oxidation, and was melted with difficulty. It was soluble in most acidic and basic media. These observations suggested a more precise identification on the basis of paper chromatography and spectrophotometric comparisons with known pterin samples.

Chromatograms of the crude pigment extracted with methanolic HCl yielded two fluorescent spots with the previous mentioned solvent mixture. The first had a pink fluorescence and an R_f value of 0.33. These results were then compared with chromatograms obtained from what is believed to be pure erythropterin (Table 2)[3]. The chromatograms of the known and unknown material were found to be identical in R_f values and in fluorescence for both sets of spots. The erythropterin's lower spot compared favorably with the value obtained by Good & Johnson (1949).

The wing pigment and erythropterin spots were analyzed spectrophotometrically, while separately and individually eluted with methanol from the paper. Strikingly similar spectra were obtained in both the visible range (350-550mμ) and in the ultra violet range (230-350 mμ) for all spots. The principle peaks were at approximately 272, 458, and 490 mμ (Table 1 and Text-figs. I & II). It should be noted that the upper and lower spots resulted in all but identical spectra, with only minor differences within the visible range. The lower erythropterin spot (Good & Johnson, 1949) is thought to be the bicyclic form, while the upper spot may be a tricyclic isomer.

A second Heliconiid, *Colaenis julia*, was investigated briefly in an attempt to determine the nature of its pigment. The spectral and chromatographic data (Table 2) obtained from this study showed that the orange color is due essentially to a pterin. The structure of this particular

[3]Both the rhizopterin and xanthopterin samples were compared in the same manner as the erythropterin and wing pigment.



TEXT-FIG. II. Ultra violet-visible spectra of the pigment extracted from *H: erato* with methanolic HCl and analyzed in a methanol solvent. Refer to Table I for numerical data for acid-base additions.

TABLE 1. ULTRA VIOLET AND VISIBLE SPECTRA* (CH_3OH solvent)

	Methanolic HCl extract	*Erythropterin*
Neutral**	273	270
	458	459
	480	481
	490	490
Plus 5% NaOH	235	235
	285	285
	462	463
	485	485
	499	498
Plus 5% HCl	272	270
	459	457
	480	...
	492	490

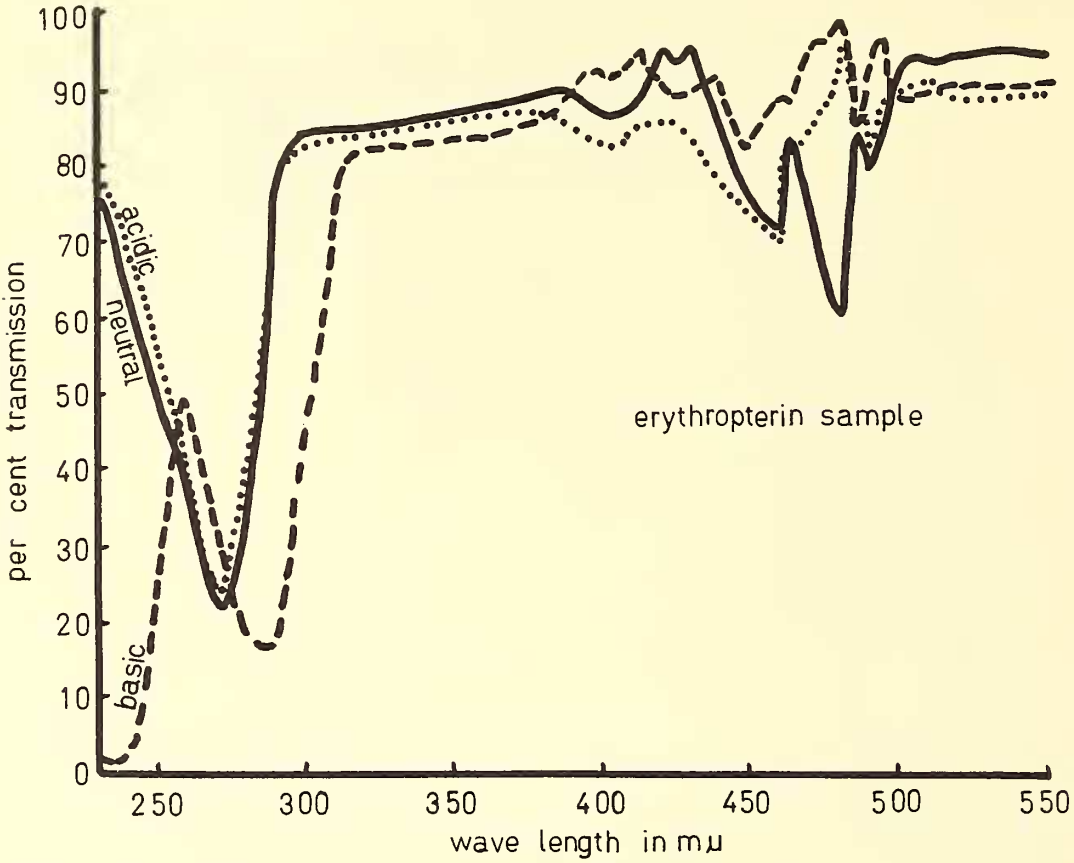
*wave length in mμ

**This is the respective order of the additions of NaOH and HCl.

TABLE 2. RELATIVE R_f VALUES OF PIGMENTS STUDIED. (Solvent–Butanol:Acetic Acid:Water, 4:1:5)

Compound	*R_f Value*
H. erato pigment (extracted with methanolic HCl)	0.10 (lower) 0.33 (upper)
Erythropterin	0.10 (lower) 0.33 (upper)
Colaenis pigment (extracted with methanol)	0.53
Rhizopterin	0.49
Xanthopterin	0.39

pterin is as yet undetermined. Both its UV and visible spectra are quite similar to those of the erythropterin and the *H. erato* extract. However, relative intensities of the individual peaks



TEXT-FIG. III. Ultra violet-visible spectra of the erythropterin sample dissolved in methanol. Refer to Table I for numerical data and for acid-base additions.

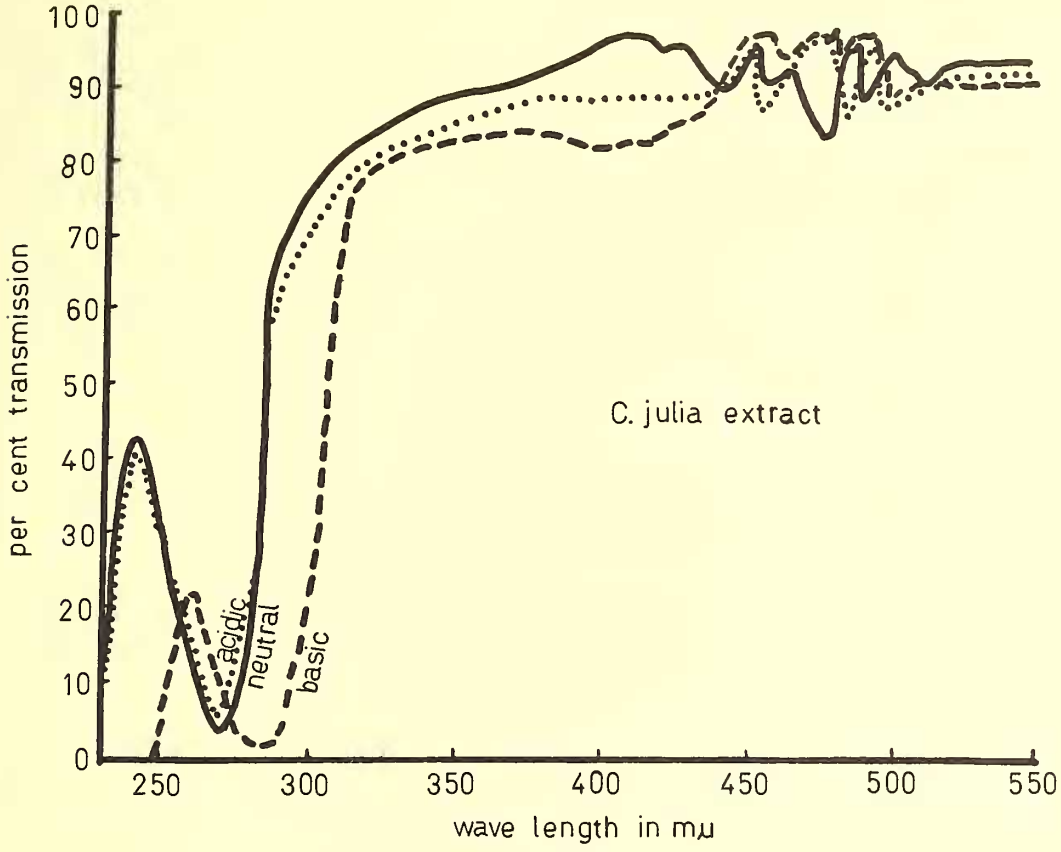
are quite different. Also, the R_f of this particular pterin is 0.55 as opposed to 0.10 and 0.33 for the erythropterin forms. Chromatographic and spectrophotometric comparisons with samples of rhizopterin and xanthopterin yielded dissimilar results.

Heliconius sara, a black Heliconiid with two yellow bands on each forewing, was also examined. Its yellow pigment is known to be a new L-∝-amino acid (Brown, 1965). However, it was checked in order to determine whether or not a pterin was also contributing to the yellow color. The resulting data were quite dissimilar to those obtained from the *H. erato* or *C. julia* specimens. The chromatographic and spectrophotometric data were identical to those of Brown and no indication of a pterin was found.

CONCLUSION

From the above data it can be concluded that erythropterin exists as a pigment within at least one Heliconiid butterfly. Also, there is no reason to doubt that it exists in other members of the same family since many have the same distinct red coloration on various portions of the body. The pigment is no doubt located on the walls of canals in the scales as it is in pierid butterflies (Ziegler-Günder, 1955).

The spectrum of the pigment extract is identical with that of the erythropterin. The fact that two spots appear on chromatograms both with the erythropterin and the methanolic HCl extract seem to indicate that this is probably not the case. There is good indication that an equilibrium exists between the two forms of this pigment. Tschesche & Barkmeier (1955) and Fieser & Fieser (1963) have suggested that erythropterin may exist in equilibrium with a tricyclic form (Text-fig. I). Excellent support for such an equilibrium is found in the fact that when individual chromatogram spots were eluted and re-run, two spots were again obtained. Both had the same R_f values and the same fluor-



TEXT-FIG. IV. Ultra violet-visible spectra of the pigment extracted from *Colaenis julia* with methanol.

escence as did the original sample. A second and less likely explanation is related to the relatively unstable nature of pterins. It might be possible to assume that certain changes in or the loss of portions of a side chain or group might cause a change in R_f without a corresponding effect upon the UV spectra (Nawa, Goto, *et al.*, 1964).

When comparing the visible spectra of Text-figures II and III, it is obvious that a discrepancy exists in regard to one of the principle peaks (480 mμ). When the neutral *H. erato* pigment is made alkaline, the 480 peak shifts out but returns upon acidification as expected. The erythropterin, however, does not do this. The 480 peak shifts out with the addition of alkali but fails to return upon acidification. This is attributed to the fact that approximately equal quantities of acid were added to the solutions of erythropterin and pigment extract. It was later realized that excess acid was needed to cause a complete re-shift.

An unidentified pterin has been detected in the orange wings of *Colaenis julia*. It possesses all the properties of pterins, has a spectra similar in shape but not intensity to that of erythropterin but has a different R_f.

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